# Effects of $La^{3+}$ on $H^{+}$ Transmembrane Gradient and Membrane Potential in Rice Seedling Roots

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**Abstract:** The effects of $LaCl_3$ on membrane potential and transmembrane proton gradient for rice (*Oryza sativa*) seedling roots were studied. Highly purified plasma membrane was isolated by aqueous two-phase partitioning method. Both the gradient of transmembrane proton and membrane potential were stimulated by certain low concentration of $LaCl_3$ and depressed by high concentration of $LaCl_3$. The optimal concentration of $La^{3+}$ is around 40～60 μmol·$L^{-1}$ for transmembrane proton gradient and membrane potential. It shows that $La^{3+}$ can influence the generations and maintenances of membrane potential and transmembrane proton gradient in rice seedling roots.

**Key words:** rare earths; rice; membrane potential; transmembrane proton gradient
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There are lots of studies in the fields of agricultural and medical applications of rare earths[1,2]. The results from China suggested that supplying rare earths might have beneficial effects on plant growth and crop products quality[3]. Some researchers demonstrated that rare earths can not enter into protoplast. Those elements are restricted out of plasma membrane[4]. It infers from the phenomenon that the plasma membrane may be the primary reaction site. The important features of plasma membrane are membrane potential and transmembrane proton gradient. The physiological role of membrane potential is to provide the driving force for active transport of ions and metabolites across plasma membrane[5]. Furthermore, transmembrane proton gradient not only provide a proton-motive force but also play an important role in energy transfer and signal transduction[6]. It is attractive to elucidate the influences of rare earths on membrane potential and transmembrane proton gradient in crops.

## 1 Experimental

Rice (*Oryza sativa* cv. Longtezao) seeds (purchased from Tonan Seeds Co., Xiamen, China) were sterilized by 0.5% sodium hypochlorite solution for 20 min, washed with flowing water, then soaked in water for 24 h and germinated in the dark at 25 ℃ for 24 h. The germinated seeds were transferred to nylon net and treated with 0, 20, 40, 60, 80, 100 μmol·$L^{-1}$ $LaCl_3$ solution and grown under the illumination of 120 μmol photons·$m^{-2}$·$s^{-1}$ for 12 h every day at room temperature for 7 days. The roots were harvested for the experiments.

Plasma membrane vesicles were prepared by two-phase partitioning method following the procedure of Sandelius et al.[7] and Zheng et al.[8]. All operations were carried out at 4 ℃.

Safranine O (purchased from Sigma), a membrane potential probe, was used in the measurements of membrane potentials. According to Schuldiner et al.[9], Rottenberg[5] and Yue et al.[10], the reaction mixture contained 250 mmol·$L^{-1}$ sucrose, 5 mmol·$L^{-1}$

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$MgSO_4$, 0.1 mmol·$L^{-1}$ FeCN, 100 mmol·$L^{-1}$ $K_3PO_4$, 0.25 mmol·$L^{-1}$ NADH, 10 μmol·$L^{-1}$ safranine O and 25 mmol·$L^{-1}$ Tris-MES (pH 7.0) in a total volume of 1.0 mL. The measurement was started by 50 μg membrane protein at 25 ℃. Fluorescence at 565 nm was recorded within 5 min by HITACHI 4010 spectrofluorometer (excitation, 495 nm). Membrane potential was expressed as the value of relative fluorescence quenching of safranine O.

Another fluorescence probe, acridine orange (purchased from Sigma) was used in the measurement of transmembrane proton gradient. According to Palmgren[11] and Yue et al[10], the reaction mixture contained 250 mmol·$L^{-1}$ sucrose, 5 mmol·$L^{-1}$ $MgSO_4$, 100 mmol·$L^{-1}$ KCl, 2 mmol·$L^{-1}$ ATP-$Na_2$, 0.25 mmol·$L^{-1}$ NADH, 0.1 mmol·$L^{-1}$ FeCN, 10 μmol·$L^{-1}$ acridine orange and 5 mmol·$L^{-1}$ Tris-MES (pH 7.0) in a total volume of 1.0 ml. The measurement was started by adding purified plasma membrane containing 50 μg protein at 25 ℃. Fluorescence at 525 nm was recorded within 5 min by HITACHI 4010 spectrofluorometer (excitation, 495 nm). Transmembrane proton gradient was expressed as the value of relative fluorescence quenching of acridine orange.

## 2 Results

**2.1 Effects of $La^{3+}$ concentration on membrane potential** Safranine O, a membrane potential fluorescence probe was used in the measurement of membrane potential for rice seedling root vesicles. The value of relative fluorescence quenching of safranine O was regarded as membrane potential. Fig. 1 shows the effects of various concentrations of $La^{3+}$ on relative fluorescence quenching of safranine O (i.e., the membrane potential). With the $La^{3+}$ concentration varied from 0 to 60 μmol·$L^{-1}$ in culture solution, the membrane potential of purified vesicles from rice seedling roots increased obviously from 5 % to around 40 %. After 60 μmol·$L^{-1}$ of $La^{3+}$, the membrane potential declined markedly. Another measurement was carried out in which the purified vesicles from the root of rice seedling grown at 0 μmol·$L^{-1}$ of $La^{3+}$ which were used and various concentrations of $La^{3+}$ added directly into the reaction medium (RM). The results show that the changes of membrane potential with a series of $La^{3+}$ additions in reaction mixture perform a similar variation compared with that in culture medium (CM). The optimal concentration of $La^{3+}$ for the membrane potential, however, becomes only 40 μmol·$L^{-1}$ in reaction medium. Fig. 1 also shows that the membrane potential of vesicles at low concentration of $La^{3+}$ (0 ~ 40 μmol·$L^{-1}$) is much higher than that of high $La^{3+}$ concentration (above 60 μmol·$L^{-1}$) in this case.

**2.2 Effects of $La^{3+}$ concentration on transmembrane proton gradient** Similar to the responses of membrane potential to various concentrations of $La^{3+}$, the changes of transmembrane proton gradient also appeared a closely correlation with various concentrations of $La^{3+}$ (Fig. 2). In the range of low $La^{3+}$ concentration (below 40 μmol·$L^{-1}$), the proton gradient across membrane increased from around 10 %~32 % for the treatment of adding $La^{3+}$ to culture solution and from around 35 % ~72 % for the treatment of adding $La^{3+}$ directly to reaction medium with the increasing of $La^{3+}$. Afterward, the proton gradient across

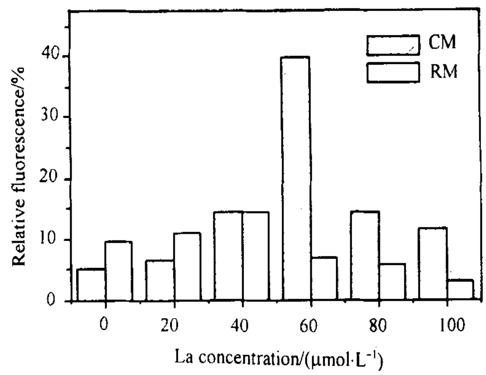


Fig. 1 Responses of membrane potential to various $LaCl_3$ concentrations in culture medium (CM) and in reaction medium (RM)

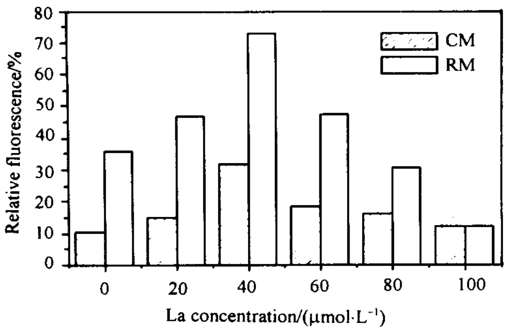


Fig. 2 Responses of transmembrane proton gradient to various $LaCl_3$ concentrations in culture medium (CM) and reaction medium (RM)

membrane both for two treatments declined with the $La^{3+}$ concentration increasing in the range from 40 to 100 $\mu mol \cdot L^{-1}$. It implies that a certain concentration of $La^{3+}$ is profitable for transmembrane proton gradient.

## 3 Discussion

The experimental results show that a proper concentration of $La^{3+}$ is beneficial for establishment and maintenance of membrane potential and transmembrane proton gradient across plasma membrane in rice seedling root. On the contrary, higher concentration of $La^{3+}$ depresses those processes. These results provide the new possibility and sight to understand and explain the concluded obtained from many studies of Chinese scientists, that is a certain concentration of rare earths has definite biological activity.

It is well known that the establishment and maintenance of membrane potential is very important to the physiological function of cell such as providing the driving force for active transport of ions and metabolites across plasma membrane[5]. The addition of various concentrations of $La^{3+}$ into culture solution can cause the changes of membrane potential for rice seedling root (Fig. 1). It implied that $La^{3+}$ can influence the tansmembrane transport of mineral nutrient elements and other biological molecules in rice seedling roots. This results suggest that there is a molecular basis for low concentration of $La^{3+}$ stimulating the uptakes of potassium, calcium, magnesium, manganese, zinc, etc. in crops concluded from Rengel[12], Diatloff et al.[13], Xiong et al.[14] and Zheng et al.[15]. The increment of nutrient elements uptake brings inevitably the improvement of crop growth. These results agree very well with the conclusion of Lao et al.[2] and Guo[16] who pointed out that a certain concentration of rare earths can stimulate the growth of crop plants and increase the yields as well.

Rottenberg[5] pointed out that there is an important contribution of tansmembrane proton gradient to membrane potential. The tansmembrane proton gradient not only provides a proton-motive force but also plays an important role in energy transfer and signal transduction[6]. Our results show that $La^{3+}$ also obviously affects the generation and maintenance of proton gradient across the plasma membrane in rice seedling root (Fig. 2). Similar to the changes of membrane potential, transmembrane proton gradient is enhanced by low concentration of $La^{3+}$ and declined by high concentration of $La^{3+}$. It also implies that a definite concentration of $La^{3+}$ can improve the uptakes of ions and stimulate some related physiological processes in rice seedling, such as acidification of cell wall in more acid apoplasm environment which is advantageous to plant cell division and expansion and finally leds to plant elongation and growth.

During measurements of transmembrane proton gradient and membrane potential, another experiment was carried out, in which only the purified plasma membrane vesicles from the control treatment of rice seedling root were used and various concentrations of $La^{3+}$ were added directly into the reaction mixture. The responses of those processes to $La^{3+}$ concentration in reaction medium case are similar to that in culture solution case. The only difference is that the optimal concentration of $La^{3+}$ for membrane potential is only 40 $\mu mol \cdot L^{-1}$ in reaction medium case, and up to 60 $\mu mol \cdot L^{-1}$ in culture solution case. This conclusion agrees very well with the results of our previous studies[8]. It can be inferred that some of $La^{3+}$ might be retarded or absorbed by cell wall of rice seedling root.

## 4 Conclusion

$La^{3+}$ influence the transmembrane proton gradient and furthermore influence the membrane potential generation and maintenance in plasma membrane of rice seedling root. A certain concentrations of $La^{3+}$ affect some physiological process, i.e., the absorption of nutrient elements and the tansmembrane transports of metabolites, even growth as well.

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## Rare Earth Metal Ion Adsorption Capacity on Cross - linked Magnet Chitosan

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**Abstract :** Magnet chitosan (MCG) was prepared with water-soluble chitosan coated by magnetite particles, which was obtained by coprecipitation of $Fe^{3+}$ and $Fe^{2+}$ in alkaline solution, and then cross-linked with glutaraldehyde. The adsorption capacity of $La^{3+}$, $Nd^{3+}$, $Eu^{3+}$, $Lu^{3+}$ ions on the magnet chitosan was studied. The effect of temperature, pH value and concentration of ions on the adsorption capacity was also investigated. It is found that the adsorption capacity of $La^{3+}$, $Nd^{3+}$, $Eu^{3+}$, $Lu^{3+}$ ions on MCG is better than on chitosan only. The highest rate of adsorption reaches 99 % on MCG. MCG can be reused for five times with high adsorption capacity. It's adsorption behavior is coincided with the equation of Langmuir.

**Key words :** rare earths; adsorption; chitoson; magnet

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